

Physiological and Morphological Responses of Soybean Culture Submitted to Applications of Glyphosate and Glyphosate + Flumioxazine

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Abstract—Soybean (*Glycine max* (L.) Merrill) is one of the most important crops in the world economy. With the official release of glyphosate- resistant transgenic soybeans in 2005, profound changes in weed management systems are occurring. The objective of the present work was to evaluate the physiological effects caused by application of EPSPS inhibitor herbicide applied in isolation and in mixture with Protox inhibitor herbicide in RR soybean plants. The experiment was conducted in the experimental area of the Federal University of Tocantins, Gurupi University Campus. The material used for planting was the cultivar SYNGENTA 1183 RR. The experimental design was a completely randomized (DIC) arranged in factorial scheme 8x6 + 1, being 8 doses of herbicides in different concentrations (4 doses of glyphosate in the dosages 480 g/ha; 980 g/ha; 1140 g/ha; 1920 g/ha plus four mixing doses of the glyphosate + flumioxazin herbicides at dosages of 480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g/ha) and the control (no application). After the application of the herbicides, 6 physiological evaluations were carried out, the first 5 days after the application and the others of 7 in 7 DAA. The parameters evaluated were: height, diameter, phytotoxicity, photosynthesis, stomatal conductance, internal carbon and transpiration. The isolated glyphosate interfered in the growth of the plants with a reduction in height in relation to the control, with increasing doses. However in the mixture there was a significant difference and in the mixture of glyphosate + flumioxazin herbicides, there was reduction in the physiological parameters.

Keywords—RR soybean, herbicides, physiology.

I. INTRODUCTION

Soy (*Glycine max* (L.) Merrill) is one of the most important crops in the world economy. According to the USDA, the world crop of this grain will be approximately 359.49 million tons. The soybean complex, composed of soybeans in grains and their derivatives, such as soybean oil and meal, was the main product exported in 2017, accounting for 14.10% of all Brazilian exports, ie US \$30.69 billion. ahead of important products such as minerals, oil, and fuels [1].

In a national senate, the main producers are Mato Grosso, Paraná, the Rio Grande do Sul and Goiás. MATOPIBA, which is composed of the states of Maranhão, Tocantins, Piauí, and Bahia, had an estimated production of 14.56 million, representing approximately

12, 3% of all national production [1].

The use of herbicides has become the most used way to control weeds in cultivated crops, however with the inadequate use of this technology, the emergence of weed species tolerant to certain herbicide molecules [2].

Currently, 480 specific cases of herbicide-resistant weed biotypes have been reported, including 251 species and 163 active ingredients, covering 91 crops distributed in 69 countries [3].

Resistance can be defined as the inherent ability of a particular weed species to survive and reproduce after exposure to a dose of lethal herbicide to the natural population. In practice, the emergence of the resistance occurs through the selection of resistant biotypes as a function of repeated and continuous application of the

same herbicide or herbicides with the same mechanism of action, during a certain period of time [2].

The RR technology allowed the use of glyphosate in post-emergence of soybean, with efficient control of species resistant to ALS and ACCase inhibitors, which were the first mechanisms to which resistant weed biotypes emerged [2].

Since it was introduced in the market, the use of glyphosate has become a frequent practice, being indicated in the control of annual and perennial weeds, being non-selective the number of applications made with glyphosate usually varies according to the herbs to be treated [4].

This herbicide, because it presents a series of advantages as the broad spectrum of action is simple to apply, does not have a residual effect on the soil present high selectivity to soybean, control species and biotypes tolerant or resistant to other mechanisms of action, low cost and more (Table 1). However, the herbicide concentration is higher than that of other herbicides in the world market [5].

The intensive use of glyphosate, a herbicide inhibitor of EPSPs, has resulted in the selection of seven resistant species: ryegrass (*Lolium multiflorum*), buva (*Conyza bonariensis*, *C. canadensis*, *C. sumatrensis*), bittergrass (*Digitaria insularis*), caruru- palmeri (*Amaranthus palmeri*) and the wing-foot grass (*Eleusine indica*) (ADEGAS, 2017).

Therefore, the use of the same herbicide or herbicides with the same action mechanism can cause problems, such as high selection pressure, increasing the possibility of selection of resistant biotypes [6] use of mixtures of herbicides.

Research has shown that the use of glyphosate combined with herbicides of different mechanisms of action has increased the spectrum and efficacy of control of plants considered more tolerant to the action of glyphosate alone [7].

Flumioxazim, which is a PROTOX inhibitor herbicide, is effective in the control of *Amaranthus viridis*, *Bidens pilosa*, *Ipomoea grandifolia* and *Portulaca oleracea*, and *Acanthosperman hispidum* and *Digitaria horizontalis* for up to 28 days after application [8].

The use of glyphosate applied alone and in combination with other herbicides is an alternative control, including herbicide resistant plants currently used in soybean cultivation. The objective of the present work was to evaluate the physiological effects caused by application of EPSPS inhibitor herbicide applied in isolation and in mixture with Prottox inhibitor herbicide in soybean plants.

II. MATERIAL AND METHODS

The experiment was conducted in the experimental area of the Federal University of Tocantins, Gurupi University Campus, located at 11 ° 43 'S, 49 ° 04' W longitude and 280 m altitude. The climate of the region is characterized as tropical climate type Aw, according to Köppen, with two well-defined seasons. The climate of the city of Gurupi according to the Thornthwaite Method Classified as C2w2A'a, hydro, evapotranspiration average annual average of 1,600 mm, distributing summer around 410 mm over the three consecutive months with higher temperature [9].

Seeding was done manually in November 2016 in plastic bags with a capacity of 5 dm³ of soil, where 4 seeds were sown per plastic bag, germination occurred 3 days after sowing, then thinning was done leaving only 1 plant per replicate. The material used for planting was the cultivar SYNGENTA 1183 RR, which is a late cycle material.

The experimental design was a completely randomized block design (DBC) of 9 treatments, (4 doses of glyphosate at dosages 480, 980 g/ha, 1140 g/ha, 1920 g/ha plus four mixing doses of glyphosate herbicides + flumioxazim at dosages of 480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g/ha) plus the control (no application). The treatments are set forth in Table 1.

The application was carried out when the plants were in the vegetative stage V4 with the aid of a costal pulverizer pressurized to carbonic gas and equipped with nozzle type nozzles (XR 110.02) with a flow of 200 L/ha and constant pressure of 35 kgf/cm². The data collection for analysis of the development of the plants was done at intervals of 7 days, in which height (H) was collected with the aid of a ruler of 50 cm, diameter (D) with the aid of a digital caliper, and for the visual analysis of phytotoxicity caused in herbicide treatments was used in percentages, where 0% = no injury and 100% = death of the crop. After application of the herbicides, the physiological evaluations were carried out, the first 5 days after the application and the others of 7 in 7 DAA,

The physiological evaluations were performed using a portable infrared gas analyzer (IRGA, model LI-6400 XT, LI-COR, inc. Lincoln, NE, USA). The evaluations were carried out between 9 and 11 am in order to maintain the homogeneous environmental conditions.

The physiological parameters evaluated were net CO₂ assimilation rate ($A - \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($g_s - \text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration rate (s^{-1}) and internal carbon ($C_i - \mu\text{mol CO}_2 \text{ mol}^{-1}$). The analyzed variables were submitted to

analysis of variance using the F test and mean test with significance of 5% probability by the Scott-Knott test

using the Sisvar program 5.6.

Table 1: Treatments evaluated and respective amounts of active ingredient, and commercial product of glyphosate and flumioxazine. (g i.a ha-1) grams of glyphosate acidequivalent in the original commercial formula Roundup @

Treatments	Commercial Product (L ha-1)	Active Ingredient (g i.a ha-1)	Stage of development
T0 - Witness	-	-	-
T1 - Glyphosate (Gly)	1,0L	480	V4
T2 - Glyphosate (Gly)	2,0L	980	V4
T3- Glyphosate (Gly)	3,0L	1140	V4
T4 - Glyphosate (Gly)	4,0L	1920	V4
T5 - Glyphosate +Flumioxazine (Gly+Flu)	1,0L	480+20	V4
T6 - Glyphosate +Flumioxazine (Gly+Flu)	2,0L	980+20	V4
T7 – Glyphosate +Flumioxazine (Gly+Flu)	3,0L	1140+20	V4
T8 - Glyphosate +Flumioxazine (Gly+Flu)	4,0L	1920+20	V4

III. RESULTS AND DISCUSSION

Table 2 shows the mean values of the height of the soybean cv. SYN 1183 RR, submitted to different doses of the herbicide glyphosate and glyphosate + flumioxazine. As shown in table 1, at 7 DAA the control differed statistically from the different concentrations of the glyphosate herbicide. Glyphosate affected the growth of soybean plants, with a reduction in height increase, relative to the control, of 13.15; 9.86; 11.74 and 10.8% at doses of (480, 980, 1140 and 1920 g.i. ha-1), respectively.

At 14 DAA only the lowest dose of glyphosate (480 g.i.a ha-1) had a statistical difference in relation to the control, with a reduction in height increase of 11.69%. The gly + flu doses obtained the lowest heights, thus a statistical difference of the control, but the lowest dose of the mixture (480 + 20 g.i.a ha-1) had the lowest reduction in height of 20.31%, differing from the other doses (980 + 20, 1140 + 20 and 1920 g.i.a ha-1) that had reductions of 42.77; 36 and 34.15%, respectively.

At 21, 28 and 42 DAA, the control group did not present statistical difference in relation to the glyphosate doses, and at 1920 DAH, the dose 1920 g.i.a ha-1 had a statistical similarity to the control, but the other doses of glyphosate alone differ statistically from with a reduction of 11.59% at dose 480 g.i.a ha-1; 17.17% at the dose 980 g.i.a ha-1 and 15.02% at the dose 1140 g.i.a ha-1. At 28 DAA, in the glyphosate (gly + flu) treatments, only the dose 480 + 20 g.i.a ha-1 was similar with the control,

while doses 980 ± 20; 1140 + 20 and 1920 + 20 g.i.a ha-1 presented statistical differences in relation to the control, corresponding to a reduction of 53.01; 45.78 and 44.06%, respectively.

At 35 DAA, a significant difference was observed in glyphosate + flumioxazine doses, but the lowest dose of gly + flu (480 + 20 g.i.a ha-1) was higher than the other doses, with a reduction of only 21.46% , followed by the doses 1140 + 22 and 1920 + 20 g.i.a ha-1 which had a smaller reduction (45.49 and 40.77%, respectively) when compared to the worst dose that was 980 + 20 g.i.a ha-1, with a reduction in height increase of 57.08%.

At 42 DAA, the lowest dose of glyphosate (480 + 20 g.i.a ha-1) did not present a statistical difference in relation to the control, but the doses 980 + 20; 1140 + 20 and 1920 + 20 g.i.a ha-1 were statistically different, but the dose 980 g.i.a ha-1 had the worst height increase with 50.88% reduction, with doses 1140 + 20 and 1920 + 20 g.i.a ha-1 had a lower reduction in height of 21.62 and 36.94% when compared to the control. Casonatto et al; (2014) [10] verified that plant height was lower in the plants that received the highest doses of glyphosate.

According to Alonso et al., (2011) [11] working with application of the glyphosate + lactofen mixture (960 + 72 g ha-1 of ia) in the V2-V3 stage of soybean cultivar CD 214 RR, promoted a reduction of 6% and 14 % at plant height at 15 and 90 DAA, respectively, when compared to

the application of glyphosate alone.

Ellis & Griffin (2003) [12] observed that treatments with glyphosate (840 and 1120 g ha⁻¹), isolated or in mixtures, with reduced rates of chlorimuron (4.5 and 6.7 g ha⁻¹), fomesafen (210 e 315 g ha⁻¹) and lactofen (112 and

168 g ha⁻¹), promoted reduction of plant height in all treatments. All stress caused to plants tends to reflect on morphophysiological changes, directly affecting their productivity [13].

Table 2: Average values of height (cm) of soybean plants cv. SYN 1183 RR, with 7, 14, 21, 28, 35 and 42 days after application (DAA) of the herbicide glyphosate (gly) in four (480 ± 980, 1140 and 1920 g ia ha⁻¹), a mixture of the herbicides glyphosate + flumioxazine (gly + flu) in four doses of 480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g ia ha⁻¹) and the control (no application). Gurupi - TO, 2017.

TREATMENTS	HEIGHT (cm)					
Days after application (AAD)	7	14	21	28	35	42
Witness	21,3 a	32,5 a	37,80 a	42,90 a	46,60 a	45,20 a
Gly (480 g.i.a. ha-1)	18,5 b	28,7 b	34,4 a	39,92 a	41,20 b	43,00 a
Gly (980 g.i.a. ha-1)	19,2 b	31,1 a	36,4 a	38,56 a	38,6 b	40,4 a
Gly (1140 g.i.a. ha-1)	18,8 b	30,8 a	35,6 a	38,62 a	39,6 b	39,8 a
Gly (1920 g.i.a. ha-1)	19,00 b	31,4 a	35,0 a	40,00 a	42,6 a	42,4 a
Gly+Flu (480+20 g.i.a. ha-1)	19,10 b	25,9 b	34,4 a	35,12 a	36,6 b	37,4 a
Gly+Flu (980+20 g.i.a. ha-1)	19,1 b	18,6 c	19,2 c	20,16 b	20,00 d	22,2 c
Gly+Flu (1140+20 g.i.a. ha-1)	19,4 b	20,8 c	24,6 b	23,26 b	25,4 c	27,00 b
Gly+Flu (1920+20 g.i.a. ha-1)	20,8 a	21,4 c	23,8 b	24,00 b	27,6 c	30,4 b
CV (%)	7,88	11,15	10,62	14,08	12,55	1,82

Means followed by the same lowercase letter in the column do not differ statistically from one another by the Scott-Knott test at 5% probability; (ns) is not significant; (*) significant at 5% and (**) significant at 1%.

Table 3 shows the mean values of the diameter (mm) of the cv. SYN 1183 RR, submitted to different doses of the herbicide glyphosate and glyphosate + flumioxazine.

At 7, 14 and 21 DAA it can be seen that the control presented a statistically higher diameter increase in relation to the different doses of glyphosate isolated, and the smallest increment of diameter at 7 DAA was observed at the 1140 g ia ha⁻¹ dose when compared to the control group, the lowest dose of glyphosate isolated (480 g ai ha⁻¹) showed the smallest increase in diameter, corresponding to a decrease of 16.42%, and at 21 days of treatment of the dose 1140 g ia ha⁻¹ obtained the smallest diameter, having a reduction of 13.59% in relation to the control.

However, at 28, 35 and 42 DAA the doses of glyphosate isolated resembled the control, not differing from each

other.

When analyzing the treatments of glyphosate + flumioxazin, it was observed that at 7 DAA the control did not present significant difference in relation to the doses of gly + flu. However, at 14, 21 and 28 DAA the control differed statistically from gly + flu doses (480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g ha⁻¹)

At 35 and 42 DAA, the lowest dose of glyphosate + flumioxazine (480 + 20 g ia ha⁻¹) was statistically similar, but the other treatments of gly + flu (980 + 20, 1140 + 20 and 1920 + 20 g ia ha⁻¹), in the two evaluation periods (35 and 42 DAA), were statically different when compared to the control, having a reduction of 27.66; 24.03 and 26.14% in the 35 DAA and 29.12; 29.88 and 28.56% at 42 DAA.

Table 3: Valores médios de diâmetro (mm) de plantas de soja cv. SYN 1183 RR, com 7, 14, 21, 28,35 e 42 dias após aplicação (DAA) do herbicida glyphosate (gly) em quatro doses (480, 980, 1140 e 1920 g.i.a ha⁻¹), mistura dos herbicidas glyphosate + flumioxazina (gly+flu) em quatro doses de (480+20, 980+20, 1140+20 e 1920+20 g.i.a ha⁻¹) e a testemunha (sem aplicação). Gurupi - TO, 2017.

Witness	DIAMETER (mm)					
Days after application (AAD)	7	14	21	28	35	42
Testemunha	4,16 a	6,82 a	7,21 a	8,66 a	8,85 a	10,71 a
Gly (480 g.i.a. ha-1)	3,56 b	5,7 b	6,32 b	7,9 a	8,66 a	10,27 a
Gly (980 g.i.a. ha-1)	3,49 b	5,74 b	6,47 b	7,97 a	8,53 a	10,62 a
Gly (1140 g.i.a. ha-1)	3,12 b	6,00 b	6,23 b	8,35 a	8,32 a	10,5 a
Gly (1920 g.i.a. ha-1)	3,58 b	5,82 b	6,26 b	7,58 a	8,5 a	9,77 a
Gly+Flu (480+20 g.i.a. ha-1)	3,88 a	5,28 c	5,35 c	7,00 b	8,11 a	9,25a
Gly+Flu (980+20 g.i.a. ha-1)	4,3 a	4,98 c	5,16 c	5,93 b	6,17 b	7,52 b
Gly+Flu (1140+20 g.i.a. ha-1)	3,94 a	5,38 c	5,11 c	6,00 b	6,48 b	7,44 b
Gly+Flu (1920+20 g.i.a. ha-1)	4,06 a	4,84 c	4,91 c	5,6 b	6,3 b	7,58 b
CV (%)	12,86	11,09	10,51	13,90	10,55	10,65

Means followed by the same lowercase letter in the column do not differ statistically from one another by the Scott-Knott test at 5% probability; (ns) is not significant; (*) significant at 5% and (**) significant at 1%.

Table 4 shows the mean values of phytotoxicity (%) of soybean cv. SYN 1183 RR, submitted to different doses of the herbicide glyphosate and glyphosate + flumioxazine.

At 7, 14, 21, 28, 35 and 42 DAA, a visual evaluation of the toxicity of soybean plants as a function of time was carried out at doses of glyphosate isolated (480, 980, 1140 and 1920 g.i.a ha⁻¹), showing no effects of the glyphosate herbicide isolated. Even at the highest dose (1920 g.i.a ha⁻¹) the visual intoxication was not noticed when compared to the control (without application), showing that the increase of the concentration of the herbicide did not affect soybean plants.

When analyzing treatments with glyphosate + flumioxazine doses (480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g.i.a ha⁻¹), when compared to the control, soybean cultivar obtained 100% of visual intoxication, thus presenting significant difference in relation to the control. When the comparison between the gly doses and the gly + flu doses, it was verified that there was a significant difference, with the doses of gly isolated were

superior 100% in relation to the doses of gly + flu in the visual intoxication of the plants of soy.

When compared between the different doses of gly + flu, the lowest dose (480 + 20 g.i.a ha⁻¹) had the lowest effect of intoxication in relation to the other doses (980 + 20, 1140 + 20 and 1920 + 20 g.i.a ha⁻¹) in all the evaluation periods (7,14,21,28,35 and 42 DAA).

Procópio et al. (2007) [7] working with isolated application of glyphosate at the doses of 480, 960 and 1440 g ha⁻¹ in RR[®] soybean plants did not verify symptoms of intoxication irrespective of the test dose isolated from glyphosate. These data do not corroborate those found by [14] Foloni et al. (2005), who observed mild intoxication effects in RR[®] soybean plants (Monsoy 8888) after isolated applications of Glyphosate.

According to Alonso et al. (2011) [15] the glyphosate + lactophene mixture caused severe visible lesions, showing symptoms similar to those observed in treatment with glyphosate + fomesafen, although with greater intensity.

Table 4: Average values of phytotoxicity (%) of soybean plants cv. SYN 1183 RR, with glyphosate (gly) herbicide at four doses (480, 980, 1140 and 1920 g/ha) at 7, 14, 21, 28, 35 and 42 days after application (DAA) flumioxazine (gly + flu) in four doses of (480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g/ha) and the control (no application). Gurupi - TO, 2017.

TREATMENTS	PHYTOXITY (%)					
Days after application (AAD)	7	14	21	28	35	42
Witness	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a
Gly (480 g.i.a. ha-1)	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a
Gly (980 g.i.a. ha-1)	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a
Gly (1140 g.i.a. ha-1)	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a
Gly (1920 g.i.a. ha-1)	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a
Gly+Flu (480+20 g.i.a. ha-1)	73,00 b	73,00 b	55,00 b	49,80 b	42,00 b	39,00 b
Gly+Flu (980+20 g.i.a. ha-1)	87,00 c	87,00 c	84,00 d	78,20 d	74,00 d	73,00 d
Gly+Flu (1140+20 g.i.a. ha-1)	84,00 c	84,00 c	73,00 c	65,40 c	66,00 c	60,00 c
Gly+Flu (1920+20 g.i.a. ha-1)	87,00 c	87,00 c	83,00 d	74,40 d	67,00 c	69,00 d
CV (%)	6,57*	6,57*	14,03*	13,77*	18,23*	14,19*

Means followed by the same lowercase letter in the column do not differ statistically from one another by the Scott-Knott test at 5% probability; (ns) is not significant; (*) significant at 5% and (**) significant at 1%.

Table 5 shows the average values of photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of cv. SYN 1183 RR, submitted to different doses of the herbicide glyphosate and glyphosate + flumioxazine.

At 7, 21, 28, 35, 42 DAA, the control compared to the glyphosate (gly) treatments alone did not show statistical difference by the Scott-Knott test at 5% probability, however the 14 DAA the control was shown statistically lower than the treatments for gly alone.

At 7 DAA, the control presented a statistical difference by the Scott-Knott test at a 5% probability, when compared to the glyphosate + flumioxazine (gly + flu) treatments, and the control was higher at 39, 47% at the 480 g/ha, 43.03% the dose 980 g/ha; 45.61% the dose 1140 g/ha; 52.16% a dose 1920 g/ha. The gly glycerol levels were higher than the gly + flu doses, and the gly-flu doses were higher than the gly + flu doses, with the Scott-Knott test at 5% probability, flu.

At 14 DAA when comparing the control with the doses of gly + flu, it was verified that there was statistical difference by the Scott-Knott test at 5%, and the control was lower than 480 + 20 g/ha and 1140 + 20 g/ha with a reduction of 34, 53% and 42.91%, respectively. However, when gly doses were compared

with gly + flu doses, the doses of gly 980 g/ha and 1920 g/ha were higher 26.56% and 40.29%, respectively with respect to gly dose + flu of 480 + 20 g/ha. With the other doses of gly and gly + flu being statistically similar.

At 28, 35 and 42 DAA, the control did not present statistical difference with the gly + flu doses, and it was the same when comparing gly doses isolated with gly + flu doses.

At 28, 35 and 42 DAA, no differences were observed between the different doses of glyphosate + flu in relation to the control, where there was also no difference when compared to the doses of gly and gly + flu.

Zobiolo et al. (2010) [16] observed that the single application (1,200 g/ha) had a greater negative effect on photosynthesis. Similar results were observed by [16] Zobiolo et al. (2010b) during the evaluation of different doses of glyphosate (600 a 2,400 g/ha) in unique applications.

Table 6 shows the mean values of the stomatal conductance ($\text{gs, mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of the cv. SYN 1183 RR, submitted to different doses of the herbicide glyphosate and glyphosate + flumioxazine

Table 5: Mean values of photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of soybean plants cv. SYN 1183 RR, with glyphosate (gly) herbicide at four doses (480, 980, 1140 and 1920 g.i.a ha⁻¹) at 7, 14, 21, 28, 35 and 42 days after application (DAA) flumioxazine (gly + flu) in four doses of (480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g.i.a ha⁻¹) and the control (no application). Gurupi - TO, 2017.

TREATMENTS	A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)					
	Days after application (AAD)					
	7	14	21	28	35	42
Witness	12,06 a	10,02 b	15,57 a	15,97 a	20,56 a	22,9 a
Gly (480 g.i.a. ha ⁻¹)	11,85 a	13,39 a	17,25 a	16,35 a	20,47 a	21,61 a
Gly (980 g.i.a. ha ⁻¹)	11,89 a	13,48 a	17,06 a	15,06 a	19,53 a	18,48 a
Gly (1140 g.i.a. ha ⁻¹)	11,91 a	14,32 a	15,49 a	13,57 a	20,56 a	22,88 a
Gly (1920 g.i.a. ha ⁻¹)	10,96 a	14,32 a	14,93 a	15,48 a	20,33 a	23,57 a
Gly+Flu (480+20 g.i.a. ha ⁻¹)	7,3 b	12,32 a	15,11 a	15,09 a	20,08 a	19,88 a
Gly+Flu (980+20 g.i.a. ha ⁻¹)	6,87 b	9,9 b	4,95 b	14,44 a	19,2 a	21,53 a
Gly+Flu (1140+20 g.i.a. ha ⁻¹)	6,56 b	11,95 a	16,94 a	14,43 a	18,81 a	20,82 a
Gly+Flu (1920+20 g.i.a. ha ⁻¹)	5,77 b	8,55 b	14,89 a	15,3 a	18,26 a	20,07 a
CV (%)	10,60*	12,14*	16,59*	20,74ns	10,94ns	14,88ns

Means followed by the same lowercase letter in the column do not differ statistically from one another by the Scott-Knott test at 5% probability; (ns) is not significant; (*) significant at 5% and (**) significant at 1%.

At 7, 14, 28, 35 and 42 the control did not differ statistically from the doses of gly isolated. However at 21 DAA the gly doses of 480, 980 and 1920 g.i.a ha⁻¹ were statistically higher than the control with an increase in GS of 61.9%, 37.09%, and 24.19%.

When comparing the gly + flu treatments to the 7 DAA with the control, it was verified that there was statistical difference by the Scott test, where the control had an increase in the photosynthetic rate of 59.05; 66.67; 72.22 and 100% corresponding to the doses of 480 980 1140 1920. When comparing the doses of gly with the doses of gly + flu, it is verified that there was significant difference, being the doses of gly superior the doses of gly + flu in the conductance.

At 14, 28, 35 and 42 DAA, the control did not present statistical difference in relation to the gly + flu doses, being the same in gly treatments isolated compared to gly + flu.

And at 21 DAA only the treatments of gly + flu at doses 980 + 20 and 1140 + 20 g.i.a ha⁻¹

1 deferred from the control, the dose 980 + 20 reduced 47.1 and dose 1140 increased 39.52% over the control. When the different doses of gly and gly + flu were related, at doses of gly 480, 980 and 1920 g.i.a ha⁻¹ were higher 35.4; Respectively, when compared to gly + flu doses 480 + 20, 980 + 20 and 1920 + 20 g.i.a ha⁻¹, and the dose of gly 1140 g ha⁻¹ is lower than 22.65% in relation to the 1140 + 20 g.i.a ha⁻¹ dose of gly + flu.

According to Magalhães Filho et al. (2008) [17], the partial stomatal closure leads to a decrease in the stomatal conductance (gs) and consequently the increase in the substomatic CO₂ (Ci).

Table 7 shows the average values of internal CO₂ concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$) of cv. SYN 1183 RR, submitted to different doses of the herbicide glyphosate and glyphosate + flumioxazine.

Table 6: Mean values of stomatal conductance (mol H₂O m⁻²s⁻¹) of cv. SYN 1183 RR, with glyphosate (gly) herbicide at four doses (480, 980, 1140 and 1920 g/ha) at 7, 14, 21, 28, 35 and 42 days after application (DAA) flumioxazine (gly + flu) in four doses of (480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g/ha)

ha-1) and the control (no application). Gurupi - TO, 2017.

TRATAMENTOS

Gs (mol H₂O m⁻² s⁻¹)

Days after application (AAD)

	7	14	21	28	35	42
Witness	0,144 a	0,087 a	0,62 b	0,863 a	0,590 a	0,793 a
Gly (480 g.i.a. ha-1)	0,136 a	0,161 a	1,00 a	1,116 a	0,665 a	0,859 a
Gly (980 g.i.a. ha-1)	0,146 a	0,156 a	0,85 a	0,978 a	0,656 a	0,764 a
Gly (1140 g.i.a. ha-1)	0,145 a	0,154 a	0,669 b	0,957 a	0,67 a	0,904 a
Gly (1920 g.i.a. ha-1)	0,131 a	0,147 a	0,771 a	0,839 a	0,652 a	0,945 a
Gly+Flu (480+20 g.i.a. ha-1)	0,059 b	0,118 a	0,646 b	1,121 a	0,619 a	0,743 a
Gly+Flu (980+20 g.i.a. ha-1)	0,048 b	0,085 a	0,328 c	1,276 a	0,608 a	0,793 a
Gly+Flu (1140+20 g.i.a. ha-1)	0,040 b	0,130 a	0,865 a	0,924 a	0,567 a	0,732 a
Gly+Flu (1920+20 g.i.a. ha-1)	0,000c	0,097 a	0,695 b	0,970 a	0,529 a	0,686 a
CV (%)	27,268	34,71ns	30,32*	33,16ns	19,53ns	27,57 ns

Means followed by the same lowercase letter in the column do not differ statistically from one another by the Scott-Knott test at 5% probability; (ns) is not significant; (*) significant at 5% and (**) significant at 1%.

At 7, 14, 21 and 28 DAA the gly doses did not present statistical difference in relation to the control, and at 35 and 42 DAA the control statistically resembled the doses of gly isolated.

At 7, 14, and 28 DAA, glyphosate + flumioxazine treatments did not differ statistically from the control, but at 21 DAA, when comparing the control with Gly + Flu doses (480 + 20; 980 + 20; 20 + 20 g/ha) only the 980 + 20 g/ha treatment presented a statistical difference when compared to the control, with a 17.02% increase in the internal CO₂ concentration.

At 35 DAA the control statistically differentiated from the gly + flu doses by the Scott-Knott test, with a reduction of the gly + flu doses, it can be seen that there was statistical difference with respect to the control, that is, all (480 + 20 g/ha) of 4.89%, while the other doses (980 + 20, 1140 + 20 and 1920 + 20 g/ha)

corresponded to a decrease of 7.32; 9.71 and 11.62% in the CO₂ Ci, respectively, relative to the control.

At 7, 14 and 28 DAA when comparing the doses of gly with the doses of gly + flu, it verified that there was no statistical difference, being that at 21 DAA only the dose 980 + 20 g/ha of gly + flu presented statistical difference, when gly doses were compared with gly + flu doses, with an increase of 13.67% over the 980 g/ha gly dose alone.

At 35 DAA the gly doses of 480, 980 and 1140 g.i.a ha-1 presented statistical differences when compared to gly + flu doses of 480 + 20, 980 + 20; 1140 + 20 g.i.a. ha-1, the gly doses being lower than the blend, with a reduction of 6.8; 3.6 and 2.77%. At 42 DAA only 480 + 20 g.i.a ha-1 differed statistically when compared to gly doses with gly + flu, being 3.67% higher than gly dose of 480 g.i.a ha-1.

Table 7: Mean values of internal CO₂ concentration ($\mu\text{molCO}_2 \text{ mol}^{-1}$) of soybean plants cv. SYN 1183 RR, with glyphosate (gly) herbicide at four doses (480, 980, 1140 and 1920 g.i.a ha⁻¹) at 7, 14, 21, 28, 35 and 42 days after application (DAA) flumioxazine (gly + flu) in four doses of (480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g.i.a ha⁻¹) and the control (no application). Gurupi - TO, 2017.

TREATMENTS	Ci (μmol		CO ₂ mol ⁻¹)			
Days after application (AAD)	7	14	21	28	35	42
Witness	207,31 a	128,91 a	211,03 b	230,85 a	257,49 a	279,45 a
Gly (480 g.i.a. ha ⁻¹)	176,26 a	199,64 a	218,37 b	224,82 a	228,25 d	271,83 b
Gly (980 g.i.a. ha ⁻¹)	188,25 a	192,36 a	213,20 b	225,52 a	230,05 d	268,69 b
Gly (1140 g.i.a. ha ⁻¹)	185,22 a	183,22 a	207,90 b	222,07 a	226,03 d	268,35 b
Gly (1920 g.i.a. ha ⁻¹)	182,12 a	171,8 a	218,33 b	218,90 a	223,75 d	264,71 b
Gly+Flu (480+20 g.i.a. ha ⁻¹)	166,58 a	176,75 a	214,8 b	233,16 a	244,9 b	282,20 a
Gly+Flu (980+20 g.i.a. ha ⁻¹)	154,55 a	149,71 a	246,94 a	237,3 a	238,65 c	273,23 b
Gly+Flu (1140+20 g.i.a. ha ⁻¹)	193,84 a	182,81 a	215,69 b	226,38 a	232,48 c	272,42 b
Gly+Flu (1920+20 g.i.a. ha ⁻¹)	198,52 a	139,12 a	215,64 b	224,00 a	227,58 d	269,79 b
CV (%)	30,23ns	36,61ns	4,72*	4,19ns	2,31*	2,16*

Means followed by the same lowercase letter in the column do not differ statistically from one another by the Scott-Knott test at 5% probability; (ns) is not significant; (*) significant at 5% and (**) significant at 1%.

The transpiration (E , mol H₂O m⁻² s⁻¹) in soybean plants as a function of the application of doses of glyphosate isolated and with the mixture (glyphosate + flumioxazine) at different times after application are described in table 8.

At 7, 21, 28, 35 and 42 DAA treatments with doses of glyphosate alone did not differ statistically from the control by the 5% probability Scott-Knott test. However at 14 DAA glyphosate doses presented a statistical difference in relation to the control, where the doses of gly isolated had an increase 54,34; 47.4; 46.24 and 42.2% in the transpiratory rate at doses 480, 980, 1140 and 1920 g.i.a ha⁻¹ when compared to the control.

When observing gly + flu treatments at 7 DAA, it was verified that the control was superior, thus presenting a significant difference between them, however, the doses 480 + 20, 980 + 20 and 1140 + 20 g.i.a ha⁻¹ excelled in relation at dose 1920 + 20

g.i.a ha⁻¹. The reduction was 47.98; 51.45 and 62.43% in doses 480 + 20, 980 + 20 and 1140 + 20 g.i.a ha⁻¹, respectively, while the highest dose (1920 + 20 g.i.a ha⁻¹) obtained the lowest E corresponding to a decrease of 99.42% in relation to the control.

At 14 DAA the dose 1140 + 20 g.i.a ha⁻¹ had a significant difference with respect to the control, having an increase of 35.84%.

The other treatments presented statistical similarity to the

control by the Scott-Knott test at 5% probability.

At 21 DAA only the treatment of glyphosate + flumioxazine (980 + 20 g.i.a ha⁻¹) presented a statistical difference in relation to the control, with a reduction of 33.90%, and those with more treatments obtained statistical similarity.

At 28, 35 and 42 DAA, all gly + flu treatments did not differ statistically when compared to the control.

At 7 DAA when comparing the doses of gly with the doses of gly + flu, it was verified that there was statistical difference between them, however the doses of gly isolated (480, 980, 1140 and 1920 g.i.a ha⁻¹) corresponding to an increase of 48.28; 53.59; 63.68 and 93.94% relative to the gly + flu doses of 480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g.i.a ha⁻¹, respectively.

At 14 DAA the gly + flu doses 480 + 20, 980 + 20 and 1920 + 20 g.i.a ha⁻¹ were lower than the doses 480, 980 and 1920 of gly, with statistical difference between them, whereas the doses of gly increased 24.72; 33.73 and 24.8% in relation to gly + flu doses (480 + 20, 980 + 20 and 1920 + 20 g.i.a ha⁻¹), and at 21 DAA only the treatment of 980 g.i.a ha⁻¹ of gly was statistically different from dose 980 + 20 g.i.a ha⁻¹, the upper dose being 40.97% in the transpiratory rate. At 28, 35 and 42 DAA the gly doses were not statistically different in relation to gly doses + flu.

Table 8: Mean values of transpiration ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of soybean plants cv. SYN 1183 RR, with glyphosate (gly) herbicide at four doses (480, 980, 1140 and 1920 g ha⁻¹) at 7, 14, 21, 28, 35 and 42 days after application (DAA) flumioxazine (gly + flu) in four doses of (480 + 20, 980 + 20, 1140 + 20 and 1920 + 20 g ha⁻¹) and the control (no application). Gurupi- TO, 2017.

TREATMENTS	E ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)					
	Days after application (AAD)					
	7	14	21	28	35	42
Witness	1,90 a	1,73 b	6,43 a	9,44 a	5,21 a	6,27 a
Gly (480 g.i.a. ha-1)	1,74 a	2,67 a	7,64 a	8,85 a	5,57 a	6,90 a
Gly (980 g.i.a. ha-1)	1,81 a	2,55 a	7,20 a	8,66 a	5,42 a	6,36 a
Gly (1140 g.i.a. ha-1)	1,79 a	2,53 a	6,69 a	8,07 a	5,40 a	7,06 a
Gly (1920 g.i.a. ha-1)	1,65 a	2,46 a	7,03 a	8,17 a	5,28 a	6,65 a
Gly+Flu (480+20 g.i.a. ha-1)	0,90 b	2,01 b	6,55 a	8,80 a	5,28 a	6,26 a
Gly+Flu (980+20 g.i.a. ha-1)	0,84 b	1,69 b	4,25 b	8,60 a	5,36 a	6,78 a
Gly+Flu (1140+20 g.i.a. ha-1)	0,65 b	2,35 a	7,24 a	8,22 a	5,18 a	6,73 a
Gly+Flu (1920+20 g.i.a. ha-1)	0,01 c	1,85 b	6,82 a	8,53 a	4,94 a	6,54 a
CV (%)	26,18*	22,03*	14,61*	13,11ns	6,77ns	10,5ns

Means followed by the same lowercase letter in the column do not differ statistically from one another by the Scott- Knott test at 5% probability; (ns) is not significant; (*) significant at 5% and (**) significant at 1%.

IV. CONCLUSIONS

Glyphosate did not significantly interfere in plant growth with a gradual reduction in height increase relative to the control, with increasing glyphosate doses both isolated and mixed.

When the phytotoxicity was evaluated, the visual intoxication was not observed, when compared to the control (without application), showing that the increase of the concentration of the herbicide did not affect soybean plants. However, the glyphosate + flumioxazine doses showed that the soybean cultivar obtained 100% intoxication, as they presented physiological damage in the soybean leaves compared to the control.

Over time in photosynthesis, stomatal conductance, transpiration and internal carbon, when compared with the control, there was no reduction of the same in the treatment with glyphosate alone.

It was observed that there is still a need for research evaluating the physiological suitability of specific soybean cultivars to determine the tolerance of the crop to different mechanisms of action in order to minimize the frequent use of herbicides and weed control strategies. good performance and productivity of the crop.

ACKNOWLEDGEMENTS

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REFERENCES

- [1] Brasil, CONAB. (2018). CONAB (National Supply Company). Follow-up of the Brazilian crop, Tenth survey, of the 2017/2018 harvest. Brasília: Ministry of Agriculture, Livestock and Animal. Welfare. 2:1–109.
- [2] ADEGAS FS, VARGAS L, GAZZIERO DLP, KARAM D, SILVA AF, et al. (2017). Impacto econômico da resistência de plantas daninhas a herbicidas no Brasil – Circular Técnica 132. EMBRAPA. 29.
- [3] I H. (2017). International survey of herbicide resistant weeds. Available. Accessed on. 14.
- [4] AMARANTE-JÚNIOR OP, SANTOS TCR, BRITO NM, RIBEIRO ML, et al. (2002). Glifosato: propriedades, toxicidade, usos e legislação. Química Nova. 25(4):589–593.
- [5] SILVA AA, SILVA JF. (2007). Topics in integrated weed management. Federal University of Viçosa. p.367.
- [6] VARGAS L, SILVA AA, BORÉM A, REZENDE ST, REZENDE ST, et al.; (1999). TOCANTINS. Secretariat of Planning and Modernization of Public Management. FERREIRA: F.A. Available from: <http://www.sefaz.to.gov.br/zoneamento/atlas-dotocantins/>.
- [7] O PS, MENEZES CCE, BETTA L, BETTA M, et al. (2007). Use of chlorimuron-ethyl and imazethapyr in Roundup Ready soybean crop. Plant weed. Plant Daninha. 25:365–373.
- [8] C DJ, M CN, M BJA, D RM, et al. (2006). Efficacy of flumioxazin, applied alone and in combination with glyphosate, for the control of weeds in citrus. Brazilian

Journal of Herbicides, Passo Fundo-RS, No. 2:45–56.

- [9] de Sousa PAB, Borges RST, Dias RR, et al.; (2012). Pesquisa e Zoneamento Ecológico-Econômico. Diretoria de Zoneamento Ecológico-Econômico - DZE. Palmas: Seplan..
- [10] S CM, et al. (2014). How glyphosate may affect transgenic soybean in different soil and phosphorus levels. Plant weed. Planta Daninha. 32:843–850.
- [11] Alonso DG, Constantin J, Oliveira Jr RS, Arantes JGZ, Cavalieri SD, et al. (2011). Selectivity of glyphosate tank mixtures for RR soybean. Weed. 29(n.4):929–937.
- [12] M EJ, L GJ. (2003). Glyphosate and broadleaf herbicide mixtures for soybean (Glycine max). Weed Technol. 17(1):21–27.
- [13] P LF, G FN, A BM, A VR, et al. (2005). Tolerância a interferência de plantas competidoras e habilidade de supressão por cultivares de soja – I. Resposta de variáveis de crescimento. Planta Daninha. 23(3):405–414. Available from: <http://dx.doi.org/10.1590/S0100-83582005000300003>.
- [14] L FL, et al. (2005). Aplicação de glifosato em pós emergência, em soja transgênica cultivada no cerrado. Revista Brasileira Herbicidas. 4:47–58.
- [15] Alonso DG, Constantin J, Oliveira Jr RS, Arantes JGZ, Cavalieri SD, et al. (2011). Selectivity of glyphosate tank mixtures for RR soybean. Planta Daninha. v.29(4):929–937.
- [16] ZOBIOLE LHS, , et al. (2011). Prevenção de injúrias causadas por glyphosate em soja RR por meio do uso de aminoácido. v. 29(1):195–205.
- [17] MAGALHÃES FILHO JR, AMARAL LR, MACHADO DFSP, MEDINA CL, MACHADO EC, et al. (2008). Water deficiency, gas exchange and root growth in 'valencia' orange tree on two types of rootstock. Bragantia, Campinas. 67(1):75–82.